

**Standard Operating Procedure for
Chlorophyll-a Sampling Method:
Field Procedure**

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1.0 Scope and Application

This method is used to filter chlorophyll-*a* samples from the Great Lakes and Tributary streams.

2.0 Summary of Method

A representative lake water sample is collected from Niskin bottles from various depths and filtered by vacuum filtration in dim light. The filter is then placed in a screw cap culture tube in the dark. The tube is stored in the dark at sub-freezing temperatures and shipped to the laboratory for extraction and analysis.

3.0 Apparatus

Plastic filter funnel, Gelman
Vacuum system (3-4 psi)
GF/F filters, Whatman (47 mm)
16 X 100 mm screw cap culture tubes
Pasteur short disposable pipets
Rubber bulb
Plastic wash bottle, 500 mL
Plastic wash bottle, 500 mL, for MgCO_3
Filter forceps
Opaque sample bottles, 500 mL (Nalgene or equivalent)

4.0 Reagents

Saturated Magnesium Carbonate Solution Add 10 grams magnesium carbonate to 1000 mL of deionized water. The solution is settled for a minimum of 48 hours. Decant the clear solution into a new container for subsequent use. *Only the clear "powder free" solution is used during subsequent steps.*

5.0 Sample Handling and Preservation

The entire procedure should be carried out as much as is possible in subdued light (green) to prevent photodecomposition. The frozen samples should also be protected from light during storage for the same reason. During the filtration process, the samples are treated with MgCO_3 solution (section 4.1) to eliminate acid induced transformation of chlorophyll to its degradation product, pheophytin. Samples are stored by station in aluminum foil and transported to a land-based laboratory in a cooler with dry ice. Analysis should be performed as soon as possible following sampling.

6.0 Field Procedure

- 6.1 Samples are provided in 500 mL opaque Nalgene bottles, labeled with the sample depth, *eg.* Surface, representing a surface sample, MI, representing the mid depth sample, or B-2, representing a bottom minus 2 meter sample.
- 6.2 Place filters, using forceps, textured side up. Assemble the filtration apparatus just prior to filtration.
- 6.3 Due to differing trophic levels among the Great Lakes, the volume of water filtered varies. For Lake Erie, 150 mLs of sample will be filtered. For Lakes Ontario, Huron, Michigan, and Superior, 250 mLs of sample will be filtered. After inverting the sample bottle several times to create a uniform mixture, carefully measure out the appropriate amount of sample using a graduated cylinder and pour contents into filtration funnel.
- 6.4 Turn on vacuum pressure on, not exceeding 3 psi.

Check Frequently During Filtration to Insure Pressure Does Not Go Above 3 PSI!!!

- 6.5 When approximately 10-50 mL of sample remains on the filter, add 10 drops of the MgCO_3 (section 4.1) solution using a disposable pipet. Thoroughly rinse the filter apparatus and graduated cylinder, using a squirt bottle, with deionized water. Turn off vacuum pressure as soon as the liquid disappears to prevent the breakage of cells.
- 6.6 Using the forceps, fold and remove the filter and carefully place it into the bottom portion of the prelabeled culture tube (see section 10) and close tightly. Lay all tubes flat and completely wrap in aluminum foil. Clearly label the Lake, station and date on masking tape and attach to above mentioned aluminum foil package. Immediately freeze. All the above procedures should be completed in subdued light.

7.0 Quality Control

The following controls are to be collected:

Control	Frequency
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Lab Dupl.	Once/batch
Field Dupl.	Once/batch
Field Blk.	Once/batch

Field blanks (Field Blk) consist of water obtained from reverse osmosis and are filtered in the same method as described in the Procedure section. A laboratory duplicate (Lab Dupl.) results when a water sample, from the same sampling bottle, is filtered twice. A field duplicate (Field Dupl.), although sampled from the same depth, is contained in a separate bottle, marked "Fld Dup".

8.0 Waste Disposal

Follow all laboratory waste disposal guidelines regarding the disposal of MgCO_3 solutions.

9.0 Shipping

Once a lake has been completely sampled for chlorophyll or a batch of 35 samples has been completed, wrap all samples into one complete batch and clearly label with survey, lake and date. Pack tightly in a medium sized cooler and fill all spaces with enough dry ice to last 24 hours. Dry ice is considered a hazardous chemical by most shipping companies and has to be accompanied by authorizing paperwork. Once receipt at CRL, the samples should be immediately placed in the freezer.

10.0 Labeling

Sample identification information is provided on printed labels both prior to and during the survey. The labels are affixed to the side of the 16x100mm chlorophyll tube. The sample identification number is covered with clear tape in case the tube becomes wet.

